

AMENDMENTS TO THE SPECIFICATION

Please amend the specification as follows:

Please insert the following paragraph and table on page 11, beginning at line 9:

“Table 1 shows the result of the determination of the iso-electric point.

Table 1

Standards	Distance (cm)	RF	pI
Carbonic anhydrase	2.2	0.20	6
β Lactoglobulin	4.7	0.42	5.3
β Lactoglobulin	5.3	0.48	5.2
Trysin inhibitor	7.1	0.64	4.5
Glucose oxidase	8.15	0.73	4.2
Amyloglucosidase	9.5	0.86	3.5
Test (activity)	3,25	0,30	5,743
Test (silver colorat.)	3,5	0,32	5,679

“

Please delete the paragraph beginning at page 11, line10.

Please delete the paragraph beginning at page 11, line 13.

Please replace the paragraph beginning at page 11, line 13, with the following amended paragraph:

“**Figure[[s 2 and 3]]1** shows the result of the determination of the iso-electric point.”

Please replace the paragraph beginning at page 11, line 15, with the following amended paragraph:

“**Figure [[4]]2** depicts a chromatogram of samples obtained by chemical saponification of *Tagetes erecta* derived lutein diesters using potassium hydroxide (**a**) and *Pleurotus sapidus* culture supernatant (**b**). Chromatogram (**c**) represents the native lutein diester pattern of marigold oleoresin.”

Please replace the paragraph beginning at page 11, line 20, with the following amended paragraph:

“**Figure [[5]]3** depicts a chromatogram (DAD, 450 nm) of samples obtained by chemical saponification of *Capsicum annuum* derived xanthophyll esters (mainly capsanthin diesters) using potassium hydroxide (a) and *Pleurotus sapidus* culture supernatant (b). Chromatogram (c) represents the native carotenoid diester pattern of paprika oleoresin.”

Please replace the paragraph beginning at page 11, line 30, with the following amended paragraph:

“The biochemical characterisation of the lipase from *P. sapidus* comprised the determination of the iso-electric point by IEF analysis by activity staining (~~Figures 1 to 3~~) and a molecular weight analysis by means of size exclusion chromatography (Table 1, Fig. 1).”

Please replace the paragraph beginning at page 13, line 31, with the following amended paragraph:

“Among the 26 commercial lipase preparations tested, with the exception of Chirazyme L-6TM (Roche Diagnostics), a lipase from *Pseudomonas sp.*, only the *Candida* (*C. rugosa*, formerly known as *C. cylindracea*, and *C. antarctica*) enzymes showed noticeable lipolytic activity towards the lutein and capsanthin esters (Table [[1]]2) (69% release of capsanthin, 44% of lutein from the respective oleoresins). Likewise, the phospholipases A1 (Lecitase NovoTM) and A2 (Lecitase 10LTM) did not show any reactivity towards xanthophyll esters. Bile salts were essential for the activity of all commercial enzymes.”

Please replace the paragraph beginning at page 14, line 3, with the following amended paragraph:

“The results obtained with the commercial enzymes are summarised in Table [[1]]2 below.”

Please replace the heading “Table 1” on page 14, line 5, with the heading “Table 2.”

Please replace the paragraph beginning at page 15, line 7, with the following amended paragraph:

“In Table ~~[[1]]~~2, preparations from genetically modified organisms are named according to the genus and species of the organism from which they were derived. The last two columns designate the amount of free capsanthin and free lutein, liberated in a standard enzymatic assay from paprika oleoresin (*Capsicum annuum* L.) or marigold oleoresin (*Tagetes erecta* L.) and are given in % in relation to the total amount, obtained after chemical saponification of a sample aliquot.”

Please replace the paragraph beginning at page 15, line 19, with the following amended paragraph:

“After six and eight culture days, respectively, solvent extracts of the mycelia and nutrient media were combined and analysed by means of HPLC with diode array detection. Significant amounts of lutein and capsanthin were liberated from their respective esters by *P. sapidus* submerged cultures. During the transformation period, the pH value shifted moderately from pH 6.65 (day 0) to pH 5.55 (day 6), indicating that enzymatic ester hydrolysis rather than pH correlated hydrolysis had occurred. The desired activity thus was detected in *P. sapidus*. The carotenoid yields after enzymatic saponification and solvent extraction did not sum to 100% (cf. Table ~~[[2]]~~3). A co-oxidative carotenoid degradation indirectly catalysed by peroxide producing enzymes may account for the losses of about 10% [Yamauchi R, Miyake N, Inoue H, Kato K Products formed by peroxy radical oxidation of β -carotene. *J. Agric. Food Chem.* 1993, **41**, 708-713].”

Please replace the paragraph beginning at page 16, line 11, with the following amended paragraph:

“The results are shown in Table ~~[[2]]~~3 below.”

Please replace the heading “Table 2” on page 16, line 13, with the heading “Table 3.”

Please replace the paragraph beginning at page 16, line 16, with the following amended paragraph:

“Conversion of xanthophyll esters derived from paprika and marigold oleoresins are given in Table [[2]] 3 in % in relation to the total amount, obtained after laboratory scale chemical saponification of a sample aliquot. As can be seen from Table [[2]] 3, enzymes obtained from *P. Sapidus* are active in the conversion of carotenoid esters to high levels of conversion, with a high specificity towards lutein and zeaxanthin esters.”

Please replace the paragraph beginning at page 17, line 26, with the following amended paragraph:

“The resulting chromatograms are shown in Figures [[4 and 5]] 2 and 3. “

Please replace the paragraph beginning at page 17, line 27, with the following amended paragraph:

“Figure [[4]] 2 shows a chromatogram (DAD, 450 nm) of samples obtained by chemical saponification of *Tagetes erecta* derived lutein diesters using potassium hydroxide (a) or *Pleurotus sapidus* culture supernatant (b). Chromatogram c represents the native lutein diester pattern of marigold oleoresin. All chromatograms are in the same scale. Peak assignment: 1 = *all-trans*-lutein, 2 = zeaxanthin. Peaks between 25 and 35 min correspond to lutein monoesters, and peaks between 38 and 45 min are lutein diesters.”

Please replace the paragraph beginning at page 17, line 33, with the following amended paragraph:

“Figure [[5]] 3 shows a chromatogram (DAD, 450 nm) of samples obtained by chemical saponification of *Capsicum annuum* derived xanthophyll esters (mainly capsanthin diesters) using potassium hydroxide (a) or *Pleurotus sapidus* culture supernatant (b). Chromatogram c represents the native carotenoid diester pattern of paprika oleoresin. All chromatograms are in the same scale. Peak assignment: 1 = *cis*-capsanthin, 2 = *all-trans*-capsanthin, 3 = zeaxanthin, 4 = β -cryptoxanthin, 5 = *all-trans*- β -carotene. Peaks between 20 and 28 min correspond to monoesters, and peaks between 35 and 45 min are diesters.”

Please replace the paragraph beginning at page 18, line 5, with the following amended paragraph:

“Under the experimental conditions applied, nearly complete ester hydrolysis was achieved with both, paprika and marigold oleoresin (Figures [[4 and 5]] 2 and 3 and Table [[2]] 3). With parallel analysed heat inactivated culture supernatants no ester cleavage was observed. The progress of liberation of carotenoids from di- via monoesterified to free compounds points to a stepwise saponification process (e.g. Figure [[4]] 2, Chromatogram b).”

Appl. No. 10/655,832
Atty. Docket No. CM2697
Amdt. dated 04/05/2004
Reply to Notice of 12/03/2003
Customer No. 27752

Amendments to the Drawings:

The attached sheets of drawings replace the original sheets of drawings filed in the instant application. Figures 1(A) and 1(B) have been deleted. Figure 2 has been deleted in favor of its insertion as Table 1 in the specification. Remaining Figures 3-5 have been renumbered beginning with Figure 1, which was formerly Figure 3, and ending with Figure 3, which was formerly Figure 5.

Attachment: Replacement Sheets